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Investigating the Effects of Exposure to Waterpipe Smoke on Pregnancy Outcomes Using an Animal Model

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Abstract

Introduction: In recent years, waterpipe tobacco smoking has been increasing in popularity all over the world. In this study, we explored effects of waterpipe smoking on pregnancy outcomes in rats.
Methods: Animals were exposed to waterpipe tobacco smoking using a whole body exposure system 2 hours per day during pregnancy. A control group was exposed to fresh air only.
Results: The results showed significant association between exposure to waterpipe smoke during pregnancy and low birth weight ($P < .01$) and neonatal death ($P < .01$). In addition, the rate of growth of offspring of the waterpipe group was significantly lower than that of control group as measured by body weight gain during the first 3 months of life ($P < .001$). No effect was found for waterpipe smoking on mean number of progeny and male to female ratio among offspring.
Conclusion: Waterpipe smoking is associated with adverse effects on pregnancy outcomes.
Implications: In this study, we investigated for the first time the effect of waterpipe smoking on pregnancy outcomes using animal model. The results clearly showed that waterpipe smoking is associated with adverse effects on pregnancy outcomes that include low birth weigh, neonatal survival, and growth retardation.

Introduction

Smoking is a major world health problem. Estimates suggest that over 5 million deaths each year are caused by tobacco use and this number may increase to over 10 million by the next two decades. The health effects of tobacco smoking are diverse and include cardiovascular diseases, lung and blood cancer and major organ diseases.[1] During pregnancy, exposure to cigarette tobacco smoke is associated with increased risk of abortion and preterm delivery, fetal growth restriction and birth defects.[2–5] Moreover, smoking during pregnancy adversely affects the health of child after birth as it is associated with high risk of developing blood cancer,[6] obesity,[7] and diabetes.[8]

Tobacco is commonly consumed in different ways including cigarette, cigar, and waterpipe (hookah, narghile, or shisha) smoking (waterpipe tobacco smoking [WTS]). While tobacco control efforts have reduced cigarette tobacco consumption significantly in many places, the use of WTS is growing throughout the world including the developed countries, especially among youth and women.[9,10] For example, in the United States, WTS is second to cigarettes as the most popular tobacco use method.[11] In some countries waterpipe

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use is more prevalent than cigarette smoking.[12,13] In addition, a significant proportion of women have been reported to use waterpipes during pregnancy. For example, 7%–9% of pregnant women in Jordan and Lebanon are current waterpipe smokers.[9,14] The spread of WTS is attributed in part to the belief that the water in the waterpipe "filters" the smoke, rendering it less harmful and less dependence-producing than cigarette smoke.[15]

In this study, we hypothesize that WTS during pregnancy are associated with adverse effects on pregnancy outcomes and future health of children born to smoker mothers. This hypothesis is based on the fact that waterpipe smoke contains similar profile of toxicants to that detected in cigarette smoke, including carbon monoxide, nicotine, and tar,[16,17] and that a previous human participant study has found an association between low birth weight and waterpipe smoking during pregnancy.[18] The hypothesis was tested using rat model and a validated whole body waterpipe smoke exposure system.[19] The results reported in the current investigation may be useful to support smoking cessation programs that target pregnant women, and to support tobacco control efforts that address WTS.

Methods

Animals and Housing

The experimental procedures were approved by the Animal Care and Use Committee (ACUC) of Jordan University of Science and Technology that follows the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023). Young adult Wistar rats weighing 230–280 g were purchased from the Animal Care Unit at Jordan University of Science and Technology (Irbid, Jordan) and used in the experiments. The rats were maintained at a 12:12 light/dark cycle and at standard temperature and air moisture. The animals were acclimated for 10 days prior to starting the mating and exposure to waterpipe smoke. Rats were given access to clean water and standard rodent food (Sahil-Huran Animal Food Company, Ramtha, Jordan). Female rats (n = 16) were randomly assigned to one of two groups; control group and waterpipe group (n = 8 each). Eight male rats were also randomly assigned to the above groups at time of mating (two females: one male—each cage).

Waterpipe Tobacco Smoke Exposure Procedure

Females in the waterpipe group were exposed to WTS for 2 hours 1 day prior to the start of mating and exposure was continued twice daily for 1 hour until delivery. Animals were exposed to WTS in a whole-body exposure chamber which was attached to a waterpipe smoking machine. The smoking machine was programmed to puff in accordance with the "Beirut Method" (171 puffs of 2.6 seconds duration, 17 seconds inter-puff interval, and 530 mL volume). The waterpipe smoke generated during each smoking machine puff is pumped into the exposure chamber (38 × 25 × 25 cm, L × W × H). During exposure sessions, the ventilation system in the exposure chamber was adjusted so that animals experienced similar levels of CO (mean ± SD, 977 ± 37 ppm). Fresh air flow into the chamber was adjusted manually to maintain a constant carbon monoxide concentration (mean ± SD, 977 ± 37 ppm) across exposure sessions, as described in our previous work.[19] Thus, the mean level of CO during exposure sessions was less than 0.1% of total gases in the chamber. During each 1 hour exposure session 10 grams of Two Apples flavor ma'assel (Nakhla brand, Egypt) was loaded in the waterpipe head. Quick-light charcoal briquettes were used as the heat source (Shaban brand, Egypt). Clean tap water was used in the waterpipe bowl and it was changed every session. Additional details about the experimental procedures are available in Khabour et al.[13,19] Female rats in the control group were exposed in the chamber to fresh air only. They were mated overnight with healthy male rats, who were also unexposed to smoke throughout the experiment.

Measurement of Pregnancy Outcomes

On the day of delivery (day 21 of gestation), the smoke exposure protocol was stopped, and thereafter the pups and dams were kept in clean air. At parturition, the pup number, the birth weight and sex of each pup was recorded. Survival of offspring and gained weight were recorded for each group to about 90 days after birth.

Statistical Analysis

Data analyses were performed using GraphPad Prism software (La Jolla, CA). Two group analyses was conducted using Student t test while multiple groups analysis was conducted using one-way analysis of variance followed by Tukey post hoc test. A P value less than .05 was considered significant.

Results

All pregnant rats from both groups completed the pregnancy until term and there were no spontaneous abortions. Exposure to WTS during pregnancy significantly decreased body weight of male and female pups in the waterpipe group (4.49 ± 0.14 g and 4.42 ± 0.12 g respectively) versus that in the control (5.86 ± 0.12 g and 6.01 ± 0.10 g, respectively), (one-way analysis of variance, F = 60.17, P < .001, Figure 1A). However, exposure to WTS during pregnancy did not affect number of male and female pups born per dam (one-way analysis of variance, F = 0.91, P = .446, Figure 1B) and male/female ratio among pups (0.91 ± 0.21 control vs. 0.86 ± 0.13 waterpipe, t = 0.204, P = .841). Pups were followed until they reached an age of 90 days. The results showed significant difference in survival after delivery (97.1% control vs. 81.9% waterpipe, P < .01, Figure 2). In addition, pups born to exposed dams gained less weight in the first 3 months following delivery compared to their counterparts in the controls (P < .001, Figure 3). Thus, exposure of rats during pregnancy significantly impacted outcomes of pregnancy.

Discussion

In this study, we reported for the first time the effects of WTS on pregnancy outcomes using an animal model. The results showed that exposure of pregnant rats to WTS is associated with decrease in birth weight and survival of pups after delivery. In addition, pups born to mothers exposed to WTS gained less weight during the first 3 months following delivery.

The adverse effects of cigarette smoking on pregnancy outcomes are well investigated. Studies using mice and whole body exposure systems have shown that maternal cigarette smoking is associated with the following adverse effects in offspring: (1) Growth retardation and low birth weight,[20] (2) Glucose intolerance and decreased brain insulin action,[21] and (3) Induction of anxiety in a novel environment, interference with spatial learning and memory formation and to sparing fear conditioning and forced swim immobility.[22] Similar findings were reported using rats model and whole body exposure to cigarette smoke.[23,24] In human studies, exposure to cigarette tobacco smoke was shown to be associated with risk of abortion and preterm delivery, fetal growth restriction and birth defects.[2,3,5] Three

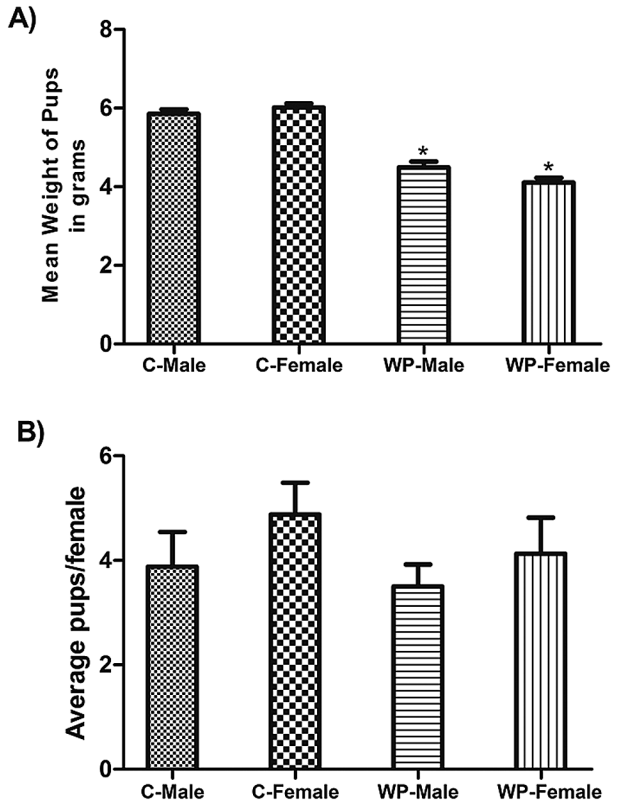

Figure 1. Effect of maternal waterpipe exposure on fetal body weight and number of born pups. Rats were exposed to waterpipe smoke for 2 hours per day during pregnancy ($n = 8$). Control group was exposed to fresh air only. (A) Exposure of pregnant rats to waterpipe smoke significantly decreased fetal body weight. (B) Exposure of pregnant rats to waterpipe smoke did not affect number of born male and female pups. *Indicate significant difference from control group ($P < .01$). C = control, WP = waterpipe, values were expressed as mean ± *SEM*.

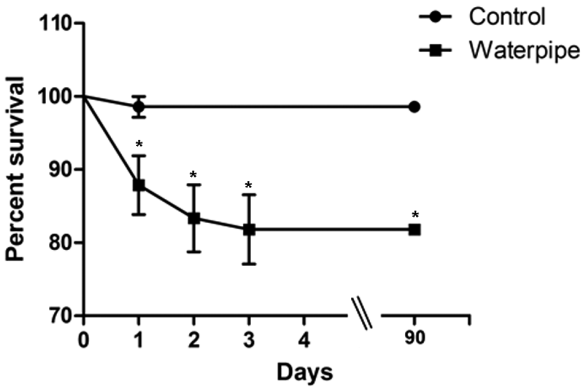

Figure 2. Survival of pups after maternal exposure to waterpipe smoke. Exposure of rats to waterpipe smoke for 2 hours per day during pregnancy significantly reduced survival of pups. *Indicate significant difference from control group ($P < .01$). Values were expressed as % ± *SD*.

studies have examined the effects of WTS on human fetal health and found that waterpipe smoking during pregnancy is associated with low birth weight.[25,26] Using an animal model, the results presented in the current study indicate that, similar to cigarette tobacco smoking, maternal exposure to WTS is associated with deleterious health effects on offspring that include low birth weight and growth retardation.

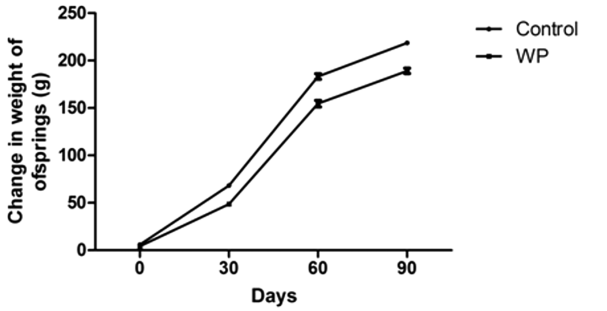

Figure 3. Growth retardation induced by maternal exposure to waterpipe smoke. Rats were exposed to waterpipe smoke for 2 hours per day during pregnancy ($n = 8$). Control group was exposed to fresh air only. Significant differences were observed between waterpipe group and control group at all time point tested. *Indicate significant difference from control group ($P < .01$). Values were expressed as mean ± *SEM*.

The results showed that number of pups born per dam is slightly lower in the waterpipe group compared to control but the difference is not statistically significant. This may imply a lack of effect for waterpipe smoking on preterm deaths among pups. This finding should be confirmed in a future study with a bigger sample size.

Previous work showed that, similar to cigarette, smoke produced by waterpipe is rich in toxicants.[16,17] For example, relative to a single cigarette, the single WTS episode yielded several folds the amount of the carcinogenic poly aromatic hydrocarbons, heavy metals and formaldehyde, eight times the carbon monoxide, and three times the nitric oxides.[27] In addition, a single waterpipe use episode is associated with 1.7 times the nicotine dose produced by consumption of single cigarette, and duration of exposure is much longer.[11,28] Prenatal nicotine exposure was shown to enhance the hypothalamic-pituitary axis and increased stress responses, which might affect the appetite of the animals.[29] In smoker men, hypothalamic-pituitary axis enhancement was documented and was associated with mode state changes.[30] Thus, it is possible that these toxic compounds play a role in the observed adverse effects of waterpipe smoke on pregnancy outcomes.[31–33] Finally, oxidative stress caused by tobacco consumption has also been shown to play a major role in the pathophysiology of smoking on the pregnancy outcomes.[34] Using mice and whole body exposure, we have previously shown that exposure to waterpipe tobacco smoke caused changes in the oxidative and inflammatory biomarkers such as GPx activity and levels of TNF-α and IL-6.[19] Similarly, exposure of mice using nose only exposure has been shown to lead to oxidative damage of the lungs.[35] Thus, multiple mechanisms might contribute to adverse effects of WTS on pregnancy outcomes.

WTS has become disturbingly popular, especially among youth and women.[10] It has been shown that a significant portion of pregnant women are either current waterpipe smokers or get exposed to waterpipe smoke at homes. In Iran, out of 2808 pregnant mothers interviewed, 8% were current waterpipe smokers and 24% were exposed to waterpipe smoke.[25] In Lebanon, about 7% of pregnant women reported smoking waterpipe during pregnancy.[14] A study by Azab et al.,[9] from Jordan showed that 8.7% of pregnant women were current waterpipe smokers while 32.8% reported exposure to secondhand waterpipe smoke at homes. Thus, WTS during pregnancy is alarming giving the high toxicant levels of the smoke. In addition, because on average, each single WTS session produces large quantities of secondhand smoke toxicants, pregnant women who share a home or workplace with a waterpipe user may be exposed to large doses of toxicants, which also might impact pregnancy outcomes.

Some parameters such as blood carboxyhemoglobin and nicotine were not measured following exposure sessions. Future studies are recommended where these parameters are measured and correlated with observed health effects of waterpipe smoking on pregnancy outcomes. This could shed light on mechanisms by which waterpipe smoking impact pregnancy outcomes.

The results presented in the current study highlight the importance of regulation of waterpipe tobacco products and waterpipe smoking cessation interventions that target pregnant women and their families.

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Declaration of Interests

None declared.

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